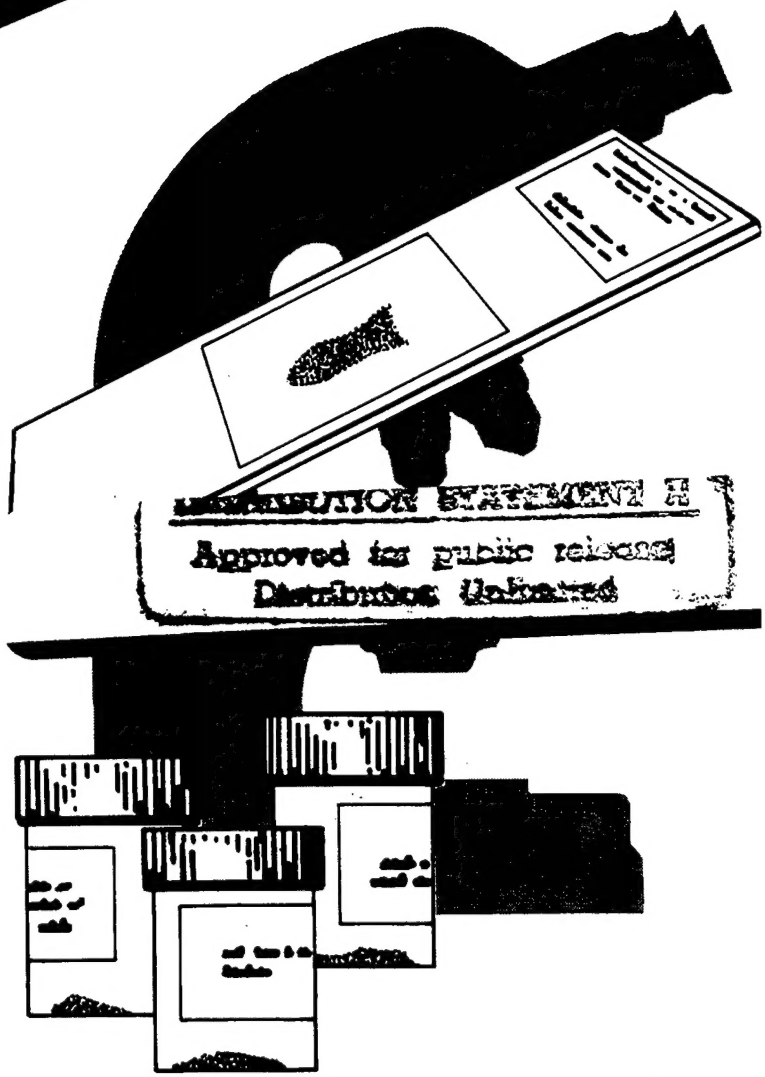
Collection and Preparation of Fish Specimens for Histological Examination



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Collection and Preparation of Fish Specimens for Histological Examination

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Histology (the study of cells) plays an important role in the accurate diagnosis of diseases of fishes, as well as those of humans and other animals. Often the state of fish health cannot be ascertained by the external appearance of the fish alone. Histology is one of many useful tools (along with microbiology, virology, immunology and other branches of science) used in disease diagnostics.

Proper preparation of fish tissue is essential for accurate histological diagnosis. It can be said that pathologists are only as good as the prepared materials they receive. These guidelines are for the preparation of fish tissue for histological examination (anatomical features mentioned are illustrated in Fig. 1). If questions arise, it is prudent to call the laboratory to which the specimens are to be sent before preparing the samples.

General Recommendations for Collecting and Preparing Specimens

1. When selecting diagnostic specimens for histological examination, select only fish that show

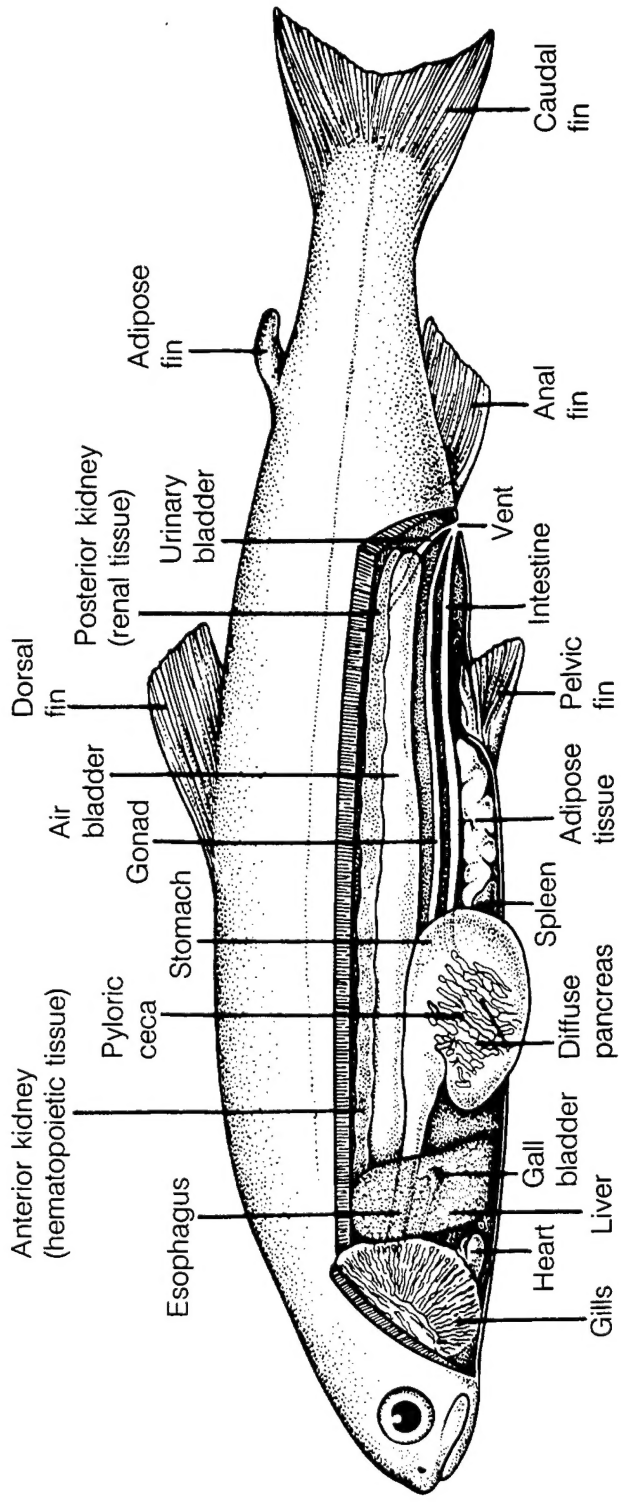

Fig. 1. Digestive tract and other anatomical features of a juvenile chinook salmon.

the typical signs of the disease in question. The fish should be moribund (in a dying state, but not dead). Fish that have been dead for longer than 10–15 min are not suitable for histological examination because post mortem changes start to take place soon after death. Place tissue or tissues in a fixing solution as soon as possible after death. Frozen fish are not desirable, because thawing causes extensive cellular damage. If possible, include normal fish of the same species from a different source. If normal fish are not available, asymptomatic fish (those not showing visible signs of disease) may be taken from the same group as the moribund fish.

2. Collect diagnostic specimens before chemical or other treatment for disease is started. Select at least five moribund and five normal (or asymptomatic) fish.
3. Label all containers, showing species and date collected.
4. Include a complete history.
   a. Source of fish
   b. Diet
   c. Disease signs and date first noticed
   d. Percent mortality (daily or weekly)
   e. Water temperature
   f. Recent handling of fish
   g. Diagram of rearing facility, showing water supply, flow, and location of affected fish
   h. List of past diseases and treatments (type, frequency, dosage)
   i. Provide any additional information that you think might be useful

Fixation of Specimens

The main objective of fixation is to precipitate the protoplasm and thus prevent further cell and tissue changes. Because the tissue decomposition process occurs soon after death, it is imperative that the specimens be placed in a fixative as soon as possible.

Fixatives

The fixative of choice is Bouin's solution; however, 10% buffered neutral formalin solution is also acceptable. Directions for preparation follow.

- Bouin's solution

  Saturated picric acid ..... 1,500 mL
  Formaldehyde, 37% solution ..... 900 mL
  Glacial acetic acid ..... 100 mL

  To prepare saturated picric acid, dissolve 21 g of picric acid in 1,000 mL of distilled water.

  Caution: Picric acid, a yellow crystalline acid, should be dampened with at least 10% distilled water for storage in the laboratory and must not be kept on the shelf in a dry form because it can be a dangerous explosive at this stage.

  Bouin's solution should be usable for at least 6 months if stored at 50–75°F.

- Buffered neutral formalin solution

  Formaldehyde, 37% solution ..... 100 mL
  Distilled water ..... 900 mL
  Sodium phosphate, monobasic, monohydrate ..... 4 g
  Sodium phosphate, dibasic, anhydrous ..... 6.5 g

  Formaldehyde, buffered neutral formalin, picric acid, and Bouin's solution can be purchased at many scientific supply companies.

Fixation Procedure

1. Kill fish by severing the spinal cord immediately behind the head and above the operculum (gill cover).
2. Slit fish ventrally along the belly, from the vent (anus) to the gills.
3. Pull viscera away from the kidney area (which is along the backbone) and puncture the air bladder to facilitate fixation of the kidney.

4. Slit the skin and body muscle on each side of the dorsal fin lengthwise, from behind the head to the tail. This cut is particularly important if the fish are longer than 40 mm (about 1½ inches).

5. Place fish or fish tissues into the fixative at a ratio of 1 part fish to 10 parts fixative.

6. Leave fish tissues in the fixing solution 24–48 h, and then transfer them to 70% ethyl alcohol (or leave them in the fixing solution if alcohol is unavailable).

7. If fish are longer than 60 mm (about 2½ inches), slit intestine along the entire length and open the cranial cap (the cartilaginous cover over the brain area) to facilitate fixation. If the fish are longer than 100 mm (about 4 inches), cut small, thin pieces of each organ (i.e., gill, heart, liver, spleen, kidney, muscle, ceca, digestive tract) and any lesion (visibly affected area). The pieces may be as large as 25 mm (about 1 inch) square, but no thicker than 5 mm (about ¼ inch).

Shipping Instructions

1. After the specimens have been in the fixative 24–48 h, or in fixative followed by alcohol, transfer them to a plastic bag, add a small amount (10–20 drops) of 70% ethyl alcohol or fixative (to keep the tissue moist), and seal.

2. The specimens can be shipped in glass bottles, but there is danger of breakage or leakage unless the materials are very well packed.

3. Box specimens in a sturdy container and ship by any convenient means.

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